REMARKS

Status of the Claims

Claims 6-9 are pending in the application. Claims 6-9 are rejected. Claim 6 is amended herein. Claims 7-9 are not amended.

The 35 U.S.C. §103(a) Rejection

Claims 6-9 are rejected under 35 U.S.C. 103(a) as being unpatentable over **Yan** *et al* (Circulation, 96(8): Suppl. P. I605, (1997), referred to as **Yan**) in view of either **Corral-Debrinski** *et al* (Mutation Research, 275: 169-180 (1992), referred to as **Corral-Debrinski I**) or **Corral-Debrinski** *et al* (JAMA, 266(13): 1812-1816 (1991), referred to as **Corral-Debrinski II**).

The Examiner finds the Applicants' response to previous office action unpersuasive and maintained the previous rejections by arguing that some of Applicants' arguments were directed to individual references and not a combination of all references. Additionally, the Examiner maintains that **Coral-Debrinski II** teaches that all of the hearts with ischemia due to atherosclerosis had much higher levels of mitochondrial DNA (mtDNA) deletion, which is

consistent with the claim of Applicants' invention. Therefore, the Examiner contends that the teachings of **Coral-Debrinski** I and II along with the teachings of **Yan** provide art recognized means for detection of DNA damage. Applicants respectfully traverse this rejection.

Claim 6 has been amended to more clearly distinguish Applicants' claimed invention from the cited prior art. Amended claim 6 recites a method of measuring the amount of oxidative stress in an individual. The method comprises the steps of collecting the tissue of interest from the individual and measuring amount of mitochondrial DNA damage in the tissue of interest. One or more of mRNA production, protein production, oxidative phosphorylation and ATP production in the mitochondria in the tissue of interest are measured where a decrease in one or more of these measurements is correlated with the mitochondrial DNA damage. Further, the amount of DNA damage in a nuclear gene in the tissue of interest is measured. Subsequently, the amount of DNA damage per length of DNA between the mitochondrial DNA and the nuclear gene is comparrf, where a greater amount of mitochondrial damage per length of DNA than nuclear DNA damage per length of DNA is

indicative of an increased amount of oxidative stress in the individual.

The specification of the instant invention teaches all the elements of the amended claim. The instant invention demonstrates a link between mitochondrial DNA damage, altered gene expression and mitochondrial dysfunction by teaching of decrease in mitochondrial DNA encoded OXPHOS gene transcripts, mitochondrial protein synthesis, cellular ATP levels and mitochondrial respiration in addition to the mitochondrial DNA damage (Example 9, page 40-48).

Additionally, the instant invention also provides the first mechanistic evidence for a role of mitochondrial dysfunction in atherogenesis by teaching that: (1) mitochondrial DNA damage is significantly increased in human and mouse atherosclerotic aorta; (2) aortic mitochondrial DNA damage increases with age *in vivo*; (3) mitochondrial DNA damage occurs *in vivo*; (3) mitochondrial DNA damage occurs prior to or is coincident with atherosclerotic lesion development *in vivo*; (4) measurement of mitochondrial DNA at an early stage provides an accurate assessment of reactive species and oxidative damage-mediated atherosclerotic risk; and (5) the putative

role of SOD2 in preventing atherogenesis in areas of turbulent flow may involve protection of the mitochondrial genome form oxidative damage (Example 20).

The Applicants contend that the combined teachings of **Yan, Corral-Debrinski** I and II do not teach all the elements of the newly amended claim 6. As discussed earlier, the instant invention teaches of an increase in mitochondrial DNA damage and a decrease in the mtDNA encoded gene transcripts (ND2 and cytochrome b), protein production, oxidative phosphorylation and ATP production. **Yan** neither teaches measuring these parameters nor does it suggest correlating them with mitochondrial DNA damage. Although both **Corral-Debrinski** I and II teach of measuring OXPHOS gene transcripts, **Corral-Debrinski** II teaches of increase in the mitochondrial DNA transcript for cytochrome b in ischemic hearts (table 3). Additionally, **Corral-Debrinski** II teaches that since analysis of hearts that failed for a variety of other reasons demonstrated increased OXPHOS gene expression irrespective of their levels of mitochondrial DNA damage, the OXPHOS gene induction may be part of general response to chronic cardiac failure (page 1815, col. 3, last para.). Thus, **Corral-Debrinski** II teaches away

from the instant invention. Therefore, if one with ordinary skill in the art were motivated to measure the amount of oxidative stress based on the combined teachings of the three cited references, one would either not correlate a decrease in mitochondrial mRNA production with mitochondrial DNA damage or one would correlate increased mitochondrial mRNA production with mitochondrial DNA damage. Neither of these would enable one of ordinary skill in the art to arrive at the instant invention.

Applicants assert that obviousness requires that the prior art relied upon fairly teach or suggest all the elements of the instant invention and that an incentive or motivation be present in the prior art to produce the claimed invention with reasonable expectation of success in its production. The Applicants have shown that the combined teachings of **Yan, Corral-Debrinski I and II** do not teach or suggest all the elements of the present invention, nor do they provide an incentive or motivation to produce the claimed invention with reasonable expectation of success in its production. Hence, the subject matter of the present invention is not obvious to one with ordinary skill in the art at the time the invention was made. Additionally claims 7 and 9 are dependent on claim 6, which cannot

be rendered obvious by **Yan, Corral-Debrinski I and II.** Accordingly, based on the above-mentioned remarks and amendments, the Applicants respectfully request that the rejection of claims 6, 7 and 9 under 35 U.S.C. 103(a) be withdrawn.

Claim 8 is rejected under 35 U.S.C. 103(a) as being unpatentable over **Yan et al.** (Circulation, 96(8): Suppl. P. I605, (1997), referred to as **Yan**) in view of either **Corral-Debrinski et al.** (Mutation Research, 275: 169-180 (1992), referred to as **Corral-Debrinski I**) or **Corral-Debrinski et al.** (JAMA, 266(13): 1812-1816 (1991), referred to as **Corral-Debrinski II**) and further in view of **Van Houten** (U.S. Pat. 5,989,816 (1999)).

The Examiner states that although **Yan, Corral-Debrinski** I or II do not specifically teach treating DNA with FAPY glycosylase prior to PCR amplification as specified in claim 8, **Van Houten** teaches the method for detection of DNA damage by detecting 8-oxo-deoxyguanosine (8-oxo-G-lesion) using FAPY glycosylase. Specifically, **Van Houten** teaches that the assay efficiently detects most forms of base damage and DNA single and double strand breaks. Further, **Van Houten** teaches that FAPY

converts the 8-oxo-dG strand break with a glycosylase/endonuclease from E.coli and the DNA was used to determine the number of lesions/17.7kb. Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the mitochondrial DNA damage methods of **Yan** in view of **Corral-Debrinski** I or II with the teachings of **Van Houten**. Applicants respectfully traverse this rejection.

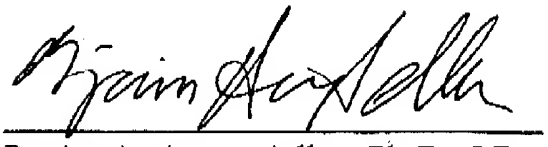
Claim 8 depends on amended claim 6. Applicants maintain that since the combined teachings of **Yan**, **Corral-Debrinski** I and II do not render claim 6 obvious, they cannot render claim 8 obvious either. Therefore, although **Van Houten** teaches the detection of DNA damage by detecting 8-oxo-deoxyguanosine using FAPY glycosylase, the combined teachings of **Van Houten** and the above cited references do not render claim 8 obvious. Accordingly, based on the above-mentioned remarks, the Applicants respectfully request that the rejection of claim 8 under 35 U.S.C. 103(a) be withdrawn.

This is intended to be a complete response to the Office Action mailed June 3, 2004. Applicants submit that the pending claims are in condition for allowance. If any issues remain

outstanding, please telephone the undersigned attorney of record for immediate resolution.

Respectfully submitted,

Date: 8/16/04



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